U.S. GEOLOGICAL SURVEY
NATIONAL WATER QUALITY LABORATORY
SOP — Laboratory Analytical Method or Procedure

SOP #	EFFECTIVE DATE:	QUALITATIVE VISUAL-SORT METHOD FOR PROCESSING BENTHIC MACROINVERTEBRATE SAMPLES
BS0332.0	April 7, 2000	
[X] New [ ] Revision	PAGE 1 of 7	
WRITTEN BY: S.R. Moulton, II and D.P. Pickard	APPROVED BY: Merle W. Shockey	

1. Scope, Application and Summary

1.1. Summary of Procedure—The goal of the qualitative visual sort processing method is to produce a comprehensive list of benthic macroinvertebrate (BMI) taxa present in a sample. This method can be applied to any BMI sample collected in the field. Samples are visually sorted by a taxonomist for up to 2 hours. Samples are first size-fractionated to separate coarse and fine organic debris to increase sorting effectiveness. The coarse size fraction is sorted for about 0.25 hour; the fine size fraction for up to 1.75 hours. Sorting targets mature, undamaged organisms that contributes to success of genus- or species-level taxonomic resolution if requested. Immature or damaged specimens are sorted from the sample only if they are to likely represent new taxa. The objective of sorting is to find as many distinct taxa as practical within the 2-hour time limit. Taxa are reported only as present; individual abundances of each taxon are not determined.

1.2. Lab codes supported by this method—2176

1.3. Reporting units and levels—Standard Taxonomic Assessment (see SOP No. BS0335.0)

1.4. Detection limits—not applicable

1.5. Interferences

1.5.1. Sorting effectiveness varies with the type and amount of sample detritus. An excessive amount of organic detritus makes it difficult to observe and remove organisms (especially small, cryptic organisms) from the sample matrix adequately.

1.5.2. Clumps of algal filaments are difficult to sort; they must be carefully separated and delicate organisms (for example, mayfly larvae) handled gently to minimize damage or loss of taxonomically valuable body parts such as gills and legs.

2. Reasons for Revision and Summary of Changes: This is a new SOP.

3. Health and Safety Warnings

3.1. Personal Safety

3.1.1. Wear long pants and closed-toed shoes at all times when working in the laboratory.

3.1.2. Wear an apron, rubber gloves, and protective eyewear during sample preparation.

3.1.3. Know the location of the nearest eyewash and shower stations.

3.1.4. Do not eat or drink in the laboratory.

3.1.5. Follow other safety procedures described in the USGS Occupational Hazards and Safety Procedures Handbook (September 1999).

3.2. Chemical Safety

3.2.1. Only work in the laboratory when the room ventilation system and fume hoods are working properly. Leave the laboratory and contact the BG supervisor if the ventilation systems are not working properly.

3.2.2. Use the preservative waste pump system to transfer preservative waste from the fume hood to the storage barrel. Contact the BG Supervisor if the system is not functioning properly. Contact the BG Safety Committee representative when the storage barrel is full and needs to be replaced.

3.2.3. Know the location of and be familiar with the Material Safety Data Sheets (MSDS) for each chemical used in the laboratory.

3.2.4. Know how to report and handle chemical and sample spills using procedures described in the NWQL Chemical Hygiene Plan (available from the Safety Program).

3.3. Follow other standard safety guidelines as describe in National Research Council (1995).

4. Sample Preservation, Handling, Containers, Analytical Processing/Holding Times, Cautions and Disposal

4.1. Each unprocessed sample is stored in a wide-mouth Nalgene™ bottle (≤ 1 L) provided by the customer. Unprocessed samples are kept in ventilated metal cabinets for each project in the BG storage facility.

4.2. Each sample received in formalin is washed and re-preserved in 70-percent ethanol within 2 weeks of receipt at the NWQL. Samples preserved in ethanol can be stored indefinitely.

4.3. Each processed sample is archived and placed in a ventilated metal cabinet located in the BG storage facility.

4.3.1. Sorted Sample Remnant

4.3.1.1. Return the sorted sample remnant to the original field container.

4.3.1.2. Place a label on the outside of the sample container with the sample ID, the name of the individual who processed the sample, and the date the sample was processed.

4.3.1.3. Add enough 70-percent ethanol to cover the remnant sufficiently and secure the container lid.

4.3.1.4. Return the sample container to the cabinet in the BG storage facility where other samples for the same project are kept.

4.3.2. Vials of Identified BMIs

4.3.2.1. After organisms have been identified from a sample, place vials in the QC cabinet on a shelf corresponding to the identifications completed in a given week.

4.3.2.2. Following QC, selected vials of identified BMI taxa will be added to the reference collection by a QC representative. All non-referenced vials are returned to the cabinet in the storage facility where other samples for the same project are kept.

4.4. Each sample remnant is disposed of according to NWQL Technical Memorandum 00.03.

4.5. Work order/worksheet handling—not applicable

SOP #	EFFECTIVE DATE:	QUALITATIVE VISUAL-SORT METHOD FOR PROCESSING BENTHIC MACROINVERTEBRATE SAMPLES
BS0332.0	April 7, 2000	
[X] New [ ] Revision	PAGE 3 of 7	
WRITTEN BY: S.R. Moulton, II and D.P. Pickard	APPROVED BY: Merle W. Shockey	

5. Preparation of Reagents/Standards/Solvents

5.1. The following chemicals are used in processing samples with this method:

5.1.1. Tap water

5.1.2. 70-percent ethanol (see SOP No. BS0331.0)

6. Apparatus

6.1. Labware

6.1.1. Forceps

6.1.2. Screw-cap vials

6.1.3. Vial racks

6.1.4. Scrub brush

6.1.5. Sorting labels

6.1.6. Plastic wash basins

6.1.7. White sorting trays of various sizes (for example, 15 x 20 cm and 20 x 30 cm)

6.1.8. Subsampling frames (see SOP No. BS0333.0)

6.2. Equipment

6.2.1. Light source (fiber-optic illuminator or portable incandescent lamp)

6.2.2. Standard metal sieves (4.75 mm mesh, plus sieve with mesh size equal to field collection mesh size)

6.2.3. Tally counter

7. Analysis

7.1. Prepare the sample according to SOP No. BS0331.0.

7.2. Size-fractionate the sample. (NOTE: If sample does not contain coarse sample matrix, bypass this step.)

7.2.1. Fill a plastic wash basin about one-third full of water.

7.2.2. Working over the wash basin, place the sample in the 4.75-mm mesh sieve. If the original sample volume is greater than 250 ml, place only a small amount (for example, one-fourth the sieve volume) of sample in the sieve.

7.2.3. Gently agitate the sieve in the wash basin to allow fine sample materials to pass through. If necessary, repeat this process until the entire sample has been size-fractionated. A properly size-fractionated sample should consist of two portions: (1) fine sample material (material passing through the 4.75-mm sieve) and (2) coarse sample material (material retained by the 4.75-mm sieve).

7.2.4. Place the coarse sample material into one or more trays for later sorting. Fill trays with enough water to cover the sample material.

7.2.5. Have a second finer mesh sieve in another wash basin then pour the fine sample material from the wash basin. The second finer mesh sieve and wash basin will recover any sample spillage.

7.3. Place small portions of the fine sample material in one or more white sorting trays. As a general guide, about 50 percent of the white bottom for each tray should be visible once the sample is evenly distributed in it.

7.3.1. If the volume of fine sample material is great enough so that more than four trays are needed, leave the fine sample material in the sieve or spread it across a gridded subsampling frame. Immerse the subsampling frame in water and cover to prevent drying.

7.3.2. Obtain small portions of the sample as needed from the sieve or subsampling frame.

7.4. Upon completion, inspect all sieves for retained organisms. Place any organisms found with the sample.

7.5. Sort the sample.

7.5.1. Total sorting time is limited to 2 hours.

7.5.2. Develop a time budget that allows at least 0.25-hour to examine the coarse sample material and evenly apportions the remaining time (up to 1.75-hours) for the fine organic sample material and elutriated inorganic debris.

7.5.3. In sample with few BMIs or with small amounts of detritus sorting may terminate before the 2-hour time limit. This action must be approved by a second taxonomist. Approval is indicated by initialing the “Sort Time” category on the BMI Identification and Enumeration Bench Data Sheet.

7.5.4. Prepare a labeled rack of vials (with screw cap lids and filled with 70-percent ethanol) corresponding to the following taxonomic grouping used to sort organisms: Gastropoda, Bivalvia, Oligochaeta, Hirudinea, Hydrachnidia, Decapoda, Amphipoda/Isopoda, Ephemeroptera, Odonata, Plecoptera, Heteroptera, Megaloptera, Trichoptera, Lepidoptera, Coleoptera, Diptera (excluding Chironomidae), Chironomidae, Other BMIs.

7.5.5. Visually sort each tray of sample by scanning left to right and top to bottom. After sorting a tray, gently rock the tray to redistribute the sample material and then quickly re-scan; remove additional organisms if necessary.

7.5.6. Sort organisms from the sample material into vials according to their taxonomic grouping. Select mature, undamaged organisms whenever possible.

7.5.7. If possible, sort at least 50 Chironomidae larvae from the entire sample.

7.5.7.1. If the sample occupies multiple trays, select larvae from each tray to obtain the number required. A tally counter is useful to keep a running total.

7.5.7.2. The objective is to maximize the number of midge taxa identified by selecting and mounting organisms with as many different combinations of diagnostic characters as possible.

7.5.7.3. All larvae are mounted on slides for samples where 50 larvae, more or less, were sorted.

7.5.7.4. Cull larvae when more than 50 larvae were originally sorted to maximize the number of different taxa mounted on slides. This step is performed using a dissecting microscope during taxonomic identification. Cull larvae for mounting on the basis of morphological characters diagnostic of common subfamilies.

	Morphological character			
Subfamily	Antennae	Ligula	Ventromental plates	Shape of head capsule
Chironominae	non-retractile	absent	well developed/ striated	round
Diamesinae	non-retractile/ annulated	absent	reduced	round/square
Orthocladiinae	non-retractile	absent	reduced	round/square
Tanypodinae	retractile	present	absent	square

7.5.8. Sort sufficient numbers of organisms for groups that are difficult to identify to genus or species visually (for example, hydropsychid caddisflies and elmid beetles).

7.5.9. Sort empty mollusk shells only if other similar looking shells do not contain soft body parts.

7.5.10. Do not sort the following organisms or life stages: vertebrates, arthropod exuvia, chironomid adults, branchiobdellids (worm-like crayfish parasites), eggs, microcrustaceans, and terrestrial organisms.

7.5.11. Consider life history information for taxa when sorting a sample that contains a range of instars and life stages. This may result in the addition of taxa to the sample data.

7.6. Clean-Up

7.6.1. Rinse and clean all sorting trays, sieves, and wash basins used to process a sample.

7.6.2. Scrub sieves with a brush and rinse from both sides to remove any entrained sample debris. Inspect each to ensure that it is clean prior to processing another sample.

7.6.3. Wipe up water and clean up workstation.

7.6.4. Put away all supplies and equipment.

7.7. Identify organisms to project-specific taxonomic levels by using the method described in SOP No. BS0335.0.

SOP #	EFFECTIVE DATE:	QUALITATIVE VISUAL-SORT METHOD FOR PROCESSING BENTHIC MACROINVERTEBRATE SAMPLES
BS0332.0	April 7, 2000	
[X] New [ ] Revision	PAGE 6 of 7	
WRITTEN BY: S.R. Moulton, II and D.P. Pickard	APPROVED BY: Merle W. Shockey	

8. Quality Control and Quality Assurance Requirements

8.1. Before starting method

8.1.1. Before preparing a sample, inspect all sieves, wash basins, and sorting trays to make sure there are no organisms or sample debris remaining from a previously processed sample.

8.1.2. Verify the sieve mesh size(s) to be used.

8.1.3. If applicable, compare the information on the internal and external sample labels; report any discrepancies to the Production Coordinator.

8.1.4. Contact the Production Coordinator immediately of problems with the sample.

8.2. Determine sorting effectiveness for each sample

8.2.1. After sorting at least 25 percent of the sample, the taxonomist asks a QC representative to scan the sample remnant for missed or under-represented BMI taxa.

8.2.2. The QC representative scans the sample remnant for about 15 minutes.

8.2.3. The QC representative may add organisms to the original series of vials.

8.2.4. The QC representative may suggest ways to improve sorting organisms from the remainder of the sample.

8.2.5. Corrective Actions

8.2.5.1. Understand any recommendation made by the QC representative.

8.2.5.2. Apply any recommendations to sorting the remainder of the sample.

8.3. Taxonomic QC—as described in SOP No. BS0335.0.

9. Data Acquisition, Calculations and Data Evaluation/Reduction—not applicable

10. Data Management and Records Management

10.1. The taxonomist records the following information in his/her laboratory record book for each sample processed:

10.1.1. Sample identification code

10.1.2. Total time to process sample

10.1.3. Problems or errors associated with the sample (for example, sample was spilled or wrong sieve was used)

10.2. Record the following information on the BMI Identification and Enumeration Bench Data Sheet

10.2.1. Circle “Qualitative”

10.2.2. Sorted by (taxonomist’s name)

10.2.3. Prep Time

10.2.4. Sort Time

11. Definitions

11.1. Visual sort—Removing organisms from a sample without the aid of magnification.

12. References

12.1. Moulton, S.R., II, Carter, J.L., Grotheer, S.A., Cuffney, T.F., and Short, T.M., 2000, Methods of analysis by the U.S. Geological Survey National Water Quality Laboratory — processing, taxonomy, and quality control of benthic macroinvertebrate samples. U.S. Geological Survey Open-File Report 00-212 (IN PRESS).

12.2. National Research Council, 1995, Prudent practices in the laboratory—Handling and disposal of chemicals: Washington, D.C., National Academy Press, 427 p.

12.3. U.S. Geological Survey, 1999, Occupational Hazards and Safety Procedures Handbook: Manual No. 445-2-h, available at http://www.usgs.gov/usgs-manual/handbook/hb/445-2-h.html

13. Key Words

benthic macroinvertebrate, qualitative sample processing, visual-sort method